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BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte JEFFREY R. SAMPSON,
JOEL MYERSON, ANNA M. TSALENKO, NICHOLAS M. SAMPAS,
PETER G. WEBB, and ZOHAR H. YAKHINI

Appeal 2007-1622
Application 09/836,012
Technology Center 1600

Decided: December 18, 2007

Before DONALD E. ADAMS, DEMETRA J. MILLS, and
LORA M. GREEN, *Administrative Patent Judges*.

MILLS, *Administrative Patent Judge*.

DECISION ON APPEAL

This appeal under 35 U.S.C. § 134 involves claims 1-17 and 74-83 the only claims pending in this application. We have jurisdiction under 35 U.S.C. § 6(b).

Representative claim follows.

1. A mixture or set of sub-mixtures comprising X-mer precursors, wherein the X-mer precursors have a minimum length of 3 nucleotides; wherein the mixture has a minimum mixture coverage complexity of at least 56/N or wherein the set of sub-mixtures has a composite mixture coverage complexity of at least 56/N, wherein N represents the number of distinct X-mer precursors in the mixture;

wherein each sub-mixture in said set has a reduced mixture coverage complexity as compared with the composite mixture coverage complexity; wherein each sub-mixture comprises a plurality of X-mer precursors; wherein said length is selected independently for each X-mer precursor; and wherein the mixture or set of sub-mixtures further comprises a set of tags that are distinguishable by mass spectrometry, wherein each tag is covalently linked to at least one X-mer precursor through a cleavable linker such that any given oligonucleotide sequence in the mixture is attached to preferably a single tag with a discrete molecular weight.

Cited References:

Southern	WO 95/04160	Feb. 9, 1995
Sorge	US 6,607,878 B2	Aug. 19, 2003
Brenner	US 5,654,413	Aug. 5, 1997

Background

According to the Specification, the

> invention relates to methods and reagents for analyzing nucleotide sequences of nucleic acids via mass spectrometry [MS], and more particularly relates to methods for analyzing nucleotide sequences employing reagents that are mixtures of oligonucleotide precursors having a high sequence-coverage complexity, and also having tags analyzable by mass spectrometry which are covalently linked to the oligonucleotides through cleavable bonds.

(Specification 1: 12-17.)

The purpose of the present invention is to determine the short word content of a target nucleic acid sequence using mass spectrometry. (Spec 9, ll. 16-17.)

> [T]he success rate of such an analysis is expected to be relatively low because of the presence of a particular mass in the mass spectrum only reveals that one of many possible nucleic acid sequences is present. For example, using only natural nucleotides, the sequence of GGCTTTA is indistinguishable by mass from the sequence of GCTTTAG, and the presence of a mass peak at 2,142 atomic mass units merely reveals that at least one of the nucleic acid sequences with 3T's, 2G's and 1A and 1C is present in the mixture.

(Specification 9: 16-23.)

A goal of the present invention is to generate either complete mixtures or sets of mixtures having releasable tags analyzable by MS where the tags utilize the available mass range of the mass spectrometer with the concomitant goal of decreasing ambiguity in data from MS analysis of nucleic acids inherent among oligonucleotides having different base-pairing patterns (sequences). (Specification 41, l. 30 to 42, l. 2.)

Grounds of Rejection

Claims 1-17 and 74-83 under 35 U.S.C. § 103(a) as obvious over Southern and Sorge.

Claims 1, 3-6, 74-80 are rejected 35 U.S.C. § 103(a) as obvious over Brenner in view of Sorge.

DISCUSSION

Claim Interpretation

Prior to consideration of the cited prior art, we interpret the pending claims. We refer to the Specification for an explanation of the concept of mixture coverage complexity claimed. A common focus of sequencing technologies is to provide methods for increasing the number of target sites that can be interrogated in a single determination where some portion of the target sequence is known (Specification 7, ll. 20-24). This is known as multiplexing (*id.*) The number of distinct oligonucleotides used in a multiplexed interrogation is generally only a small subset of the theoretical sequence-complete set. (Specification 7, ll. 27-30). This ratio of actual sequence coverage provided by a particular oligonucleotide mixture to the theoretical coverage provided by the sequence-complete set is defined as the "mixture coverage complexity." (Specification 7: 27-32.) For example, "for 8-mer probes, the sequence complete set has 4^8 or 65,536 members. If the number of interrogation sites in the multiplexed determination is about 500 . . . then the mixture coverage complexity would be equal to 500/65,536 or approximately 1/130." (Specification 8, ll. 5-10.)

If the mixture has a set of submixtures of probes, "the minimum mixture coverage complexity (CC_M) of the mixture (or minimum composite mixture coverage complexity of the set of submixtures) is 56/N, where N is the number of distinct X-mers in the mixture." (Spec. 10, ll. 12-14.) Each submixture in the set has a reduced mixture coverage complexity relative to the composite mixture coverage complexity. (Specification 10, ll. 7-19.)

With this understanding of "mixture coverage complexity" we review the claims in view of the cited prior art.

Obviousness

Claims 1-17 and 74-83 under 35 U.S.C. § 103(a) as obvious over Southern and Sorge.

Claim 1 covers embodiments to both mixtures and submixtures of X-mer precursors. We focus our attention on the mixture embodiment of claim 1. Thus, claim 1 requires the following elements with respect to mixtures.

- X-mer precursors having a minimum length of 3 nucleotides

-The mixture has a minimum mixture coverage complexity of at least 56/N

-The length is selected independently for each X-mer precursor

-The mixture comprises a set of tags that are distinguishable by mass spectrometry, wherein each tag is covalently linked to at least one X-mer precursor through a cleavable linker such that any given oligonucleotide sequence in the mixture is attached to preferably a single tag with a discrete molecular weight.

The Examiner finds

> Southern et al. teach a composition (mixture) of claims 1-2, 7, 12, 14, 81-82, comprising X-mer precursor having a minimum length of 6 nucleotides (see page 5, line 28-36, page 55, line 13-23, page 2, line 27-33), wherein the mixture the mixture [*sic*] has at least complexity of at least 56/N, wherein N represents the number of distinct X-mers (see page 5, line 28-36, Fig. 3a, page 55, line 13-23); wherein the mixture comprises a set of tags (reporter groups) and each tag is covalently linked to at least one X-mer through a cleavable linkage (see page 6, paragraph 2, page 7, line 3-6, page 14, line 1-24).

(Answer 3.)

In addition, Southern discloses that the tag moiety consists of one or more reporter groups distinguishable by mass and thus capable of being analyzed by mass spectrometry. (Southern 2, ll. 33 to 3, ll. 21.) In one embodiment of Southern the analyte is coupled to a nested set of reporter structures designed to make it easier to deduce the structure of the analyte from the composition of the tag. (Southern 6, ll. 36-35.) The reporter groups could take many forms, the main consideration is the need to read the composition or sequence of the tag by mass spectrometry. (Southern 7:3-6.) Possibilities include different atomic weights or formula weights, such as aliphatic chains of different lengths or different isotopic composition. (Southern 7, ll. 3-12.) Southern further discloses that each analyte of the mixture is labeled with a unique tag. (Southern 5: 15-36.)

The Examiner relies on Sorge as further teaching tags distinguishable by mass spectrometry. (Answer 4.) Sorge teaches a

> collection of uniquely tagged molecules wherein Sorge teach the use of oligonucleotide tags, each with discrete molecular weight (see col. 22, line 56-67, col. 23, line 1- 6). Sorge also teaches that the use of molecular weight tags would allow for unambiguous identification of molecular weight after cleavage and provide sequence information of each DNA fragment in a given restriction pattern and identification of any particular nucleotide sequence in the mixture (see col. 23, line 1-22). Sorge also teaches tags that are distinguishable by mass spectrometry and a kit comprising said mixture of DNA fragments (see col. 27, line 10-67).

(Answer 4.)

The Examiner concludes that

> [i]t would have been prima facie obvious to a person of ordinary skill in the art at the time the invention was made to modify the mixture of X-mer precursors as taught by Southern et al. with a step of using tags with discrete molecular weight as taught by Sorge for the purpose of enriching tagged precursors by targeting size differences in oligonucleotide tags to provide an efficient sorting of DNA fragments in a mixture. One skilled in the art would be motivated to combine the mixture of x-mers as taught by Southern et al. with the inclusion molecular weight tags as taught by as taught by Sorge because Sorge explicitly taught that the use of molecular weight tags would allow for unambiguous identification of molecular weight after cleavage and provide sequence information of ach DNA fragment in a given restriction pattern and identification of any particular nucleotide sequence in the mixture…

(Answer 4-5.)

We agree that the Examiner has provided sufficient evidence to support a prima facie case of obviousness.

In particular, Southern teaches

- X-mer precursors forming a library consisting of 4^n reagents (Southern 3: 25-31) and an array of 4^8 or 65,536 octanucleotides (Southern 19, 29-34) or 4^6 or 4096 hexanucleotides (Southern 5: 27-34).

-The mixture has a minimum mixture coverage complexity of at least 56/N. Southern discloses a complete set of hexamers having 4096 possible combinations of nucleotides. Thus, Southern teaches a coverage complexity that is at least 56/N. Specifically, Southern teaches 4096/4096 = 1 > 56/4096.[1]

[1] Appellants' Specification provides examples of mixture coverage complexity which meet the 56/N requirement and examples which do not meet the 56/N requirement. Examples of mixtures

-The length of the X-mer precursor is selected independently for each X-mer precursor in the hexanucleotide mixture described by Southern. (Southern 5: 28-34.)

-The mixture comprises a set of tags that are distinguishable by mass spectrometry, wherein each tag is unique and may be covalently linked to at least one X-mer precursor through a cleavable linker such that any given oligonucleotide sequence in the mixture is attached to preferably a unique tag with a discrete molecular weight. (Southern 5: 28-30; 14: 1-30.)

Thus the prior art, Southern, discloses a method of detection of analytes of interest using "a core molecule with the ability to vary various substitutent groups . . . in a combinatorial manner with mass spectrometry

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having the specifications described above, by way of illustration and not limitation, include; (1) a mixture Ω_1 consisting of 60 of the possible 64 3-mers (CCm (Ω_1) = 15/16, which is greater than 56/60 = 14/15); (2) a mixture Ω_2 consisting of 128 of the possible 256 4-mers (CCm (Ω_2) = 1/2, which is greater than 56/128 = 7/16); (3) a mixture Ω_3 consisting of 256 of the possible 1,024 5-mers (CCm (Ω_3) = 1/4, which is greater than 56/256 = 7/32); (4) a mixture Ω_4 consisting of 512 of the possible 4,096 6-mers (CCm (Ω_4) = 1/8, which is greater than 56/512 = 7/64); (5) a mixture Ω_5 consisting of 1,024 of the possible 4,096 6-mers (CCm (Ω_5) = 1/4); (6) a mixture Ω_6 consisting of 48 5-mers and 512 6-mers (CCm(Ω_6) = 11/64); (7) a mixture Ω_7 consisting of 128 5-mers, 512 6-mers and 128-7mers (CCm (Ω_7) = 33/128); (8) a mixture Ω_8 consisting of 256 5-mers, 1,000 6-mers and 96 7-mers (CCm (Ω_8) = 1/2).

Examples of mixtures that do not conform to the above specifications, by way of illustration and not limitation include; (1) a mixture Ω_9 consisting of 64 of the possible 256 4-mers ((CCm (Ω_9) = 1/4 < 56/64), (2) a mixture Ω_{10} consisting of 128 of the possible 1,024 5-mers ((CCm (Ω_{10}) = 1/8 < 56/128), (3) a mixture Ω consisting of 384 6-mers and 128-7mers ((CCm(Ω_{11}) = 13/128 < 56/512), (4) a mixture consisting of 64 5-mers, 256 6-mers and 64 7-mers ((CCm (Ω_{12}) = 33/256 < 56/384). (Specification 29, l. 20 to 30, l. 4.)

tags." (Southern 2: 18-21.) The tag consists of one or more reporter groups distinguishable by mass and capable of being analyzed by mass spectrometry. (Southern 2: 34-36.) Analysis of the tag moieties indicates the nature of the analyte moieties bound to the target substance. (Southern, Abstract.) Southern further describes a method of sequencing nucleic acids which employs a library of reagents to determine the sequence of a target nucleic acid. (Southern, Abstract.) Sorge similarly describes the use of tags for identifying combinatorial synthesis molecules. (Sorge Abstract; col. 22, l. 56 to col. 23, l. 6.)

Sorge employs unique lengths of nucleic acid tags such that any number of molecules can be identified simultaneously and accurately without prior separation of each of the tags and or molecules. (Sorge, col. 6, ll. 30-39.) Each weight or length will encode not only the identity of the building blocks used to make each molecule in the library, but also the order of synthesis used to make the molecule. (Sorge, col. 6, ll. 28-39.) In one embodiment four different colors correspond to four possible bases at a given nucleotide position. (Sorge, col. 23, ll. 14-16.)

We find that it would have been obvious for one of ordinary skill in the art to substitute the unique mass spectrometry tags of Southern with the mass spectrometry tags of Sorge to discern information about the analyte X-mer.

Appellants argue,

> there must be a teaching in the relevant art which would suggest to a person having ordinary skill in that art the desirability of combining the "ladder tag" design of Southern, where each discrete oligonucleotide sequence within the mixture is associated with a "spectrum" of mass entities, with

> the molecular weight "blocks" of colors or other tags of Sorge, where certain molecular weight ranges or "gaps" are reserved for post-digestion analysis. There is no teaching in either reference that would suggest the desirability of combining.

(Br. 5.)

We note that the Supreme Court has recently emphasized that "the [obviousness] analysis need not seek out precise teachings directed to the subject matter of the challenged claim, for a court can take account of the inferences and creative steps that a person of ordinary skill in the art would employ" (*KSR Int'l v. Teleflex Inc.*, 127 S. Ct. 1727, 1741, (2007)). In the present case Southern teaches a reagent comprising an analyte linked to a reported group adapted for detection by mass spectrometry. (Abstract, Col. 2, ll. 8-25.) In Southern, the tag or reporter moiety provides information about the nature of the analyte. (Southern 2, ll. 21-36.) Thus the disclosure of Southern alone provides motivation to label each structural variant with a unique tag discernable by mass spectrometry. (Southern 5, ll. 22-24.)

Sorge's methodology also permits the identification of a large subset of target molecules out of a very large collection of similar or dissimilar molecular species. (Sorge, col. 5, ll. 55-60.) The method is used to create tagged molecules that identify any collection of molecular species, including amino acids and nucleotides. (Sorge, col. 5, ll. 55-65.) We find that one of ordinary skill in the art familiar with the methodology of Southern using a reporter group analyzed by mass spectrometry would have been motivated to use molecular weight blocks of colors or other molecular weight tags of Sorge to provide information about the nature of a unique analyte.

"[W]hen the question is whether a patent claiming the combination of elements of prior art is obvious" the relevant question is "whether the improvement is more than the predictable use of prior art elements according to their established functions." *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1740, (2007). In addition, "[w]hen there is a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product ... of ordinary skill and common sense." (Id. at 1742.)

In the present case, the Examiner has shown with appropriate evidence that it was known in the art to use mass spectrometry tags to provide useful information about the nature of an analyte (amino acids and nucleotides). One known tag detectable by mass spectrometry was described by Sorge. This tag could be physically cleaved away from the oligonucleotides made in combinatorial synthesis before molecular weight analysis. (Sorge, col. 34, ll. 12-15.) Thus, Sorge provides one possible predictable solution option available to one of ordinary skill in the art to detect amino acids or nucleotides without prior separation of each tag or molecule.

Appellants further argue that Southern does not specifically teach a mixture or set of sub-mixtures of the independent claims, wherein the mixture has a minimum mixture coverage complexity of at least 56/N. (Br. 5-6.)

The Specification, page 29, provides examples of mixture coverage complexity which meet the 56/N requirement and examples which do not meet the 56/N requirement.

As indicated above, Southern discloses a complete set of hexamers having all 4096 possible combinations of nucleotides. Thus, Southern's complete set of hexamers meets the claim limitation of a minimum mixture coverage complexity of at least 56/N, as claimed.

After evidence or argument is submitted by the Applicant in response to an obviousness rejection, "patentability is determined on the totality of the record, by a preponderance of evidence with due consideration to persuasiveness of the argument." *In re Oetiker*, 977 F.2d 1443, 1445, (Fed. Cir. 1992). We find the preponderance of the evidence weighs in favor of a finding of obviousness of the claimed subject matter and the obviousness rejection is affirmed.

Obviousness

Claims 1, 3-6, 74-80 are rejected 35 U.S.C. § 103(a) as being unpatentable over Brenner in view of Sorge.

The Examiner finds that

> Brenner teaches a composition (mixture) of claims 1, 3-5, comprising X-mer precursor having a minimum length of 3 nucleotides (see col. 3, line 15-67, col. 4, line 1-8, col. 7, line 39- 60), wherein the mixture the mixture has at least complexity of at least 56/N, wherein N represents the number of distinct X-mers (see col. 7, table II shows complexity of at least 56/N); wherein the mixture comprises a set of tags and each tag is covalently linked to at least one X-mer through a cleavable

> linkage (see col. 9, line 25-67, col. 10, line 1-67, col. 11, line 1-65).

(Answer 5.)

The Examiner acknowledges that Brenner does not specifically teach any oligonucleotide sequence in the mixture is attached to preferably a single tag with a discrete molecular weight, or tags distinguishable by mass spectrometry. (Answer 6.) The Examiner again relies on Sorge to meet this limitation. (Answer 6.)

The Examiner concludes that

> [i]t would have been prima facie obvious to a person of ordinary skill in the art at the time the invention was made to modify the mixture of x-mer precursors as taught by Brenner with a step of using tags with discrete molecular weight that are distinguish-able by mass spectrometry as taught by Sorge for the purpose of enriching tagged precursors by targeting size differences in oligonucleotide tags to provide an efficient and sensitive sorting of DNA fragments in a mixture.
>
> One skilled in the art would be motivated to combine the mixture of X-mers as taught by Brenner with the inclusion molecular weight tags as taught by Sorge because Sorge explicitly taught that the use of molecular weight tags would allow for unambiguous identification of molecular weight after cleavage and provide sequence information of each DNA fragment in a given restriction pattern and identification of any particular nucleotide sequence in the mixture (see col. 23, line 1-22). Sorge also explicitly taught tags that are distinguishable by mass spectrometry (see col. 27, line 10-67). An ordinary artisan would have a reasonable expectation of success that inclusion of discrete molecular weight tags that are distinguishable by mass spectrometry would result in enriching sequence information of any oligonucleotide of interest in the mixture and sensitivity of the detectable tags in identifying the sequence information, and such modification of the X-mer mixture would be obvious over the cited prior art …

(Answer 6-7).

We again find that the Examiner has presented sufficient evidence to support a prima facie case of obviousness.

Brenner teaches

- X-mer precursors having 3 or 4 nucleotides (col. 3, line 15-67, col. 4, line 1-8, col. 7, ll. 45-60; col. 18, ll. 5-16).

-The mixture has a minimum mixture coverage complexity of at least 56/N. The Examiner points to col 7, Table II of Brenner as evidencing a mixture coverage complexity of at least 56/N. We do not find the incomplete 4-mer set of table II meets the at least 56/N requirement. A complete set of 4-mers would be represented by 4^4, or 256, molecules. Table II of Brenner identifies 107 molecules. 107/256 = .42. 56/107 = .52. .42 < .52 thus the 4-mer set of Brenner's table II does not have a coverage complexity of at least 56/N.

However, Brenner also discloses an oligonucleotide set comprising "every possible 3-mer" which meets the at least 56/N requirement (col. 18, ll. 5-16). A complete set of 3-mers would be represented by 4^3, or 64. 64/64 = 1. 56/64 - .87. 1 > .87, thus Brenner's 3-mer set does have a coverage complexity of at least 56/N.

-The length of the 3-mer is selected independently for each X-mer precursor

-The mixture comprises labels (col. 9, ll. 25 to col. 10, l. 20).

We agree with the Examiner's reasoning and analysis in support of a prima facie case of obviousness of Brenner in view of Sorge.

Appellants contend that they claim X-mer precursors linked to tags and Brenner refers only to oligonucleotide tags, and therefore does not disclose the claimed X-mer linked to a tag. (Br. 10.) The Examiner contends that Appellants definition of X-mer precursor as set forth in the Specification page 16 does not exclude an oligonucleotide tag from being considered an X-mer precursor. (Answer 11.)

The Specification page 16 defines "X-mer precursors" as

> a nucleic acid sequence that is complementary to a portion of the target nucleic acid sequence. The oligonucleotide precursors are sequences of nucleoside monomers joined by phosphorus linkages *(e.g.,* phosphodiester, alkyl and aryl-phosphate, phosphorothioate, phosphotriester), or non-phosphorus linkages (*e.g.,* peptide, sulfamate and others). They may be natural or synthetic molecules of single-stranded DNA and single-stranded RNA with circular, branched or linear shapes and optionally including domains capable of forming stable secondary structures (*e.g.,* stem-and-loop and loop-stem-loop structures). The oligonucleotide precursors contain a 3'-end and a 5'-end.

The tags of Brenner comprise subunits consisting of oligonucleotides having a length of three to six nucleotides which are hybridized to their respective complements (col. 4, ll. 1-8) and which may be used for DNA sequencing (col. 1, ll. 35-40). The oligonucleotides may be labeled with fluorescent dyes (col. 19, ll. 15-18) or attached to reactive functionalities through a linking group (col. 9, l. 25 to col. 10, l. 9). We agree with the Examiner that the oligonucleotides of Brenner meet Appellants definition of X-mer precursors, as claimed.

Appellants further argue that Brenner fails to teach an X-mer precursor having a mixture coverage complexity of at least 56/N. (Br. 9.)

As discussed above, we find the 3-mer oligonucleotide tags of Brenner meet the "at least 56/N" mixture coverage complexity limitation.

Thus we find Appellants have failed to rebut the Examiner's prima facie case of obviousness, and the obviousness rejection is affirmed.

CONCLUSION

The rejection of claims 1-17 and 74-83 under 35 U.S.C. § 103(a) as obvious over Southern and Sorge is affirmed.

The rejection of claims 1, 3-6, and 74-80 under 35 U.S.C. § 103(a) as obvious over Brenner in view of Sorge is affirmed.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

AFFIRMED

Ssc:

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